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**Asynchronous hatching provides females with a means for
increasing male care but incurs a cost by reducing
offspring fitness**

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ABSTRACT

In species with biparental care, sexual conflict occurs because the benefit of care depends on the total amount of care provided by the two parents while the cost of care depends on each parent's own contribution. Asynchronous hatching may play a role in mediating the resolution of this conflict over parental care. The sexual conflict hypothesis for the evolution of asynchronous hatching suggests that females adjust hatching patterns in order to increase male parental effort relative to female effort. We tested this hypothesis in the burying beetle *Nicrophorus vespilloides* by setting up experimental broods with three different hatching patterns: synchronous, asynchronous and highly asynchronous broods. As predicted, we found that males provided care for longer in asynchronous broods whereas the opposite was true of females. However, we did not find any benefit to females of reducing their duration of care in terms of increased lifespan or reduced mass loss during breeding. We found substantial negative effects of hatching asynchrony on offspring fitness as larval mass was lower and fewer larvae survived to dispersal in highly asynchronous broods compared to synchronous or asynchronous broods. Our results suggest that, even though females can increase male parental effort by hatching their broods more asynchronously, females pay a substantial cost from doing so in terms of reducing offspring growth and survival. Thus, females should be under selection to produce a hatching pattern that provides the best possible trade-off between the benefits of increased male parental effort and the costs due to reduced offspring fitness.

Key words: sexual conflict, parental care, asynchronous hatching, *Nicrophorus vespilloides*

INTRODUCTION

Sexual conflict, defined as a divergence in the evolutionary interests of individuals of the two sexes (Parker, 2006), is now recognised as ubiquitous in a variety of contexts, including mating and parental care (Arnqvist & Rowe, 2005). In species with biparental care, there is conflict over how much care each parent should provide because the benefit of care depends on the total amount of care provided by the two parents while the cost of care depends on each parent's own contribution (Lessells, 2012). As a consequence of this conflict, each parent is expected to minimise its costs of care by shifting as much of the workload as possible over to its partner (Trivers, 1972). One mechanism that might play a role in mediating the resolution of sexual conflict over parental care is asynchronous hatching, which occurs when the offspring from a single reproductive event hatch over an extended period of time (Clark & Wilson, 1981). The sexual conflict hypothesis for the evolution of asynchronous hatching suggests that females adjust hatching patterns in order to increase male parental effort relative to female effort (Slagsvold & Lifjeld, 1989). Female birds can control hatching patterns by altering the timing of the onset of incubation: the brood hatches synchronously if the onset of incubation occurs after the clutch has been completed, while it hatches asynchronously if incubation begins before the last egg has been laid (Clark & Wilson, 1981). The initial version of this hypothesis proposed that the female benefits from hatching the young more asynchronously by making the male start feeding the young as soon as the earliest offspring hatch such that he continues feeding for longer than with a synchronous brood (Slagsvold & Lifjeld, 1989). However, a later version (sometimes termed the 'exploitation of mate hypothesis'; Slagsvold *et al.*, 1995), suggested that the female benefits from hatching the young more synchronously because the male has to contribute more effort to prevent the brood from starving due to the peak in demand of each offspring occurring simultaneously.

The hypothesis that asynchronous hatching plays a role in mediating the resolution of sexual conflict over parental care predicts that the female should gain a fitness benefit from adjusting hatching patterns by increasing her partner's contribution towards parental care,

thereby allowing her to reduce her own contribution. Previous studies on birds, which have tested this hypothesis by manipulating the degree of hatching asynchrony, have found mixed evidence. In support of the exploitation of mate hypothesis, Slagsvold (1997) found that males contributed more care towards synchronous broods while females reduced their contribution relative to asynchronous broods. Other studies have been unable to detect a consistent difference in male feeding rate between synchronous and asynchronous broods (Hillström, 1992; Amundsen, 1993; Hébert & Sealy, 1993; Stoleson & Beissinger, 1997). Furthermore, Slagsvold *et al.* (1994) found that synchronous hatching increased female survival in the subsequent year while asynchronous hatching increased male survival. In contrast, Stoleson & Beissinger (1997) found no difference in survival of male and female parents raising synchronous or asynchronous broods. Asynchronous hatching and biparental care are not unique to birds, but also occur in some insects (Nalepa, 1988; Müller & Eggert, 1990) and reptiles (While *et al.*, 2007). Thus, to improve our understanding of how asynchronous hatching contributes towards the resolution of sexual conflict over parental care, there is now a need to extend this work to non-avian systems.

Burying beetles of the genus *Nicrophorus* exhibit hatching asynchrony similar to that of many altricial birds (Müller & Eggert, 1990; Smiseth *et al.*, 2006; Takata *et al.*, 2015). These beetles breed on small vertebrate carcasses, which are buried underground (Scott, 1998). Females lay eggs in the surrounding soil (Pukowski, 1933). In contrast to birds, burying beetles do not incubate the eggs. Instead, the asynchronous hatching pattern is determined by the period of time over which the eggs are laid, which is termed 'laying spread' (Smiseth *et al.*, 2006; Takata *et al.*, 2015) and the extent to which laying is skewed towards the earlier part of the laying period, which is termed 'laying skew' (Smiseth *et al.*, 2008). Thus, females can control the hatching pattern simply by adjusting laying spread and laying skew. In *Nicrophorus vespilloides* (Herbst), the mean interval between the hatching of the first and last larvae of a brood (i.e., hatching spread) is 30 hours. Given that the larvae disperse into the soil around 6 days after hatching, the hatching spread is considerable relative to the amount of time the larvae spend on the carcass (Smiseth *et al.*, 2006).

Nicrophorus vespilloides exhibits facultative biparental care (Wilson & Fudge, 1984). Either parent is capable of raising the brood alone, providing the opportunity for one parent to desert the brood and leave the other to care for the offspring (Bartlett, 1988). Parents provide care by preparing the carcass, defending it and the brood from predators and conspecifics, applying antimicrobials to the carcass, and provisioning the larvae with pre-digested carrion (Eggert *et al.*, 1998; Rozen *et al.*, 2008; Walling *et al.*, 2008; Arce *et al.*, 2012). Sexual conflict over parental care occurs if parents benefit from reducing their investment in the current brood by increasing their survival and future reproductive success or by increasing the chances of finding another mate during the breeding season (Maynard Smith, 1977). *Nicrophorus vespilloides* appears to fulfil these criteria because there is a cost associated with providing care (Ward *et al.*, 2009) and both sexes can breed more than once in a season (Bartlett & Ashworth, 1988) without any delay after rearing a brood (Scott & Traniello, 1990).

We conducted two experiments to test the sexual conflict hypothesis in *N. vespilloides*. Previous work on the resolution of sexual conflict over parental care highlights the distinction between evolutionary and facultative responses when studying how a focal parent adjusts its care to a change in the partner's workload, termed 'sealed-bids' and 'negotiation', respectively (Lessells 2012). Thus, in Experiment 1, we tested whether females facultatively adjust hatching patterns in order to manipulate males to increase their contribution to parental care. Given that biparental care in *N. vespilloides* is facultative, females may adjust hatching patterns depending on whether the male partner is present or absent at the start of breeding. The presence or absence of the male might provide females with a reliable cue as to whether a male is likely to assist in providing care for the larvae once the eggs have hatched. We recorded the timing of oviposition of females laying in the presence or absence of a male using scanners to minimise interference while females lay eggs. If females facultatively adjusted hatching patterns, we predicted that laying spread would differ when the male was present compared to when he was removed. In Experiment 2, we tested whether variation in the hatching pattern influences the male's contribution

towards parental care and whether there is a benefit to females should the male make a greater contribution to parental care. Burying beetles do not differentiate between their larvae and larvae produced by other females as long as the larvae are introduced after their own eggs have hatched (Müller & Eggert, 1990). This allows us to use a cross-fostering design where we provided females with foster broods of a standardised brood size and a particular degree of asynchrony. We set up broods with three different hatching patterns (synchronous, asynchronous and highly asynchronous broods) and recorded how long each parent remained with the brood as a proxy for the amount of parental care. We assessed the fitness consequences for the parents by measuring effects on the survival and growth of the larvae and on the longevity and mass change of the parents. If asynchronous hatching plays a role in mediating the resolution of sexual conflict over parental care, we predicted that females would reduce their duration of care in broods with a greater hatching spread, with a corresponding increase in male care. We expected that reducing the amount of effort they invest in parental care would lead to a fitness benefit for females, such as an increase in the female's lifespan or a reduction in her loss of body mass during breeding.

METHODS

Study animals

The beetles used in this study were from an outbred laboratory population maintained at the University of Edinburgh. Beetles were housed individually in clear plastic boxes (124 x 82 x 22 mm or 110 x 110 x 33 mm). They were kept at $20 \pm 2^{\circ}\text{C}$ (mean \pm range) under constant lighting and were fed small pieces of organic beef twice a week. The beetles were aged 18-27 days post-eclosion at the start of the experiments.

Experimental Procedures

Experiment 1

To determine whether females facultatively adjust laying patterns to increase the male's contributions towards care, we allowed females to lay eggs either in the presence or the

absence of a male. We paired unrelated virgin males and females and placed them in a clear breeding box (17cm x 12cm x 6 cm) containing 1cm of compost. We supplied each pair with a mouse carcass weighing 19.56-22.27g (previously frozen, supplied from Livefoods Direct Ltd, Sheffield, UK), which is within the range of vertebrate carcasses utilised by beetles in the wild (range: 1-37g; Müller *et al.*, 1990; Smiseth & Moore, 2002). We removed the male from half of the boxes after 6 hours, while leaving the male with the female in the remaining boxes (male present n=26, male absent n=24). Previous work suggests that parents respond to the absence of their partner within 45 minutes of removal (Steiger & Müller, 2010). Thus, given that the first eggs were laid after an average of 24 hours after pairing, females had ample time (on average 18 hours) to notice the male's absence before they began oviposition. Eggs are visible at the bottom of the breeding box and can be seen on images obtained by placing the boxes on flat-bed scanners (Canon Canoscan 9000F Mark II). In the small amount of soil used, the visible number of eggs is very similar to the actual clutch size (Monteith *et al.*, 2012). We scanned the breeding boxes every hour using Vuescan professional edition software (Hamrick Software) until after the completion of oviposition. From the scanned images, we counted the number of new eggs laid each hour to determine the laying spread (the time between the first and last egg being laid) and the clutch size. We calculated a laying skew index (based on the hatching skew index of Smiseth *et al.*, 2008) for each brood using the following formula: $\sum((t_i - t_m)/t_m)p_i$, where p_i is the proportion of the total clutch laid each hour, t_i is the time interval starting from the initiation of oviposition and t_m is the middle of the laying period. To account for possible effects due to female and male body size, we also measured the pronotum widths of the parents using a Mitutoyo Absolute Digimatic calliper with a precision of 0.01mm. We set up 86 pairs initially but in analyses we excluded all pairs where either the eggs did not hatch (n=26) or there were technical problems with the scanner (n=10).

Experiment 2

In order to investigate the influence of hatching patterns on the duration of care provided by

males and females, we used a 2x3 fully factorial design with male presence versus absence and hatching spread (synchronous, asynchronous or highly asynchronous hatching) as the main factors. To set up the broods, we weighed virgin beetles, paired females with unrelated males, and placed each pair in a clear breeding box (17cm x 12cm x 6cm) containing 1-2cm of compost. We provided each pair with a mouse carcass weighing 19.37-22.22g (previously frozen, supplied from Livefoods Direct Ltd, Sheffield, UK). In half of the trials, we removed the male 6 hours after we provided the pair with a carcass, which is before the female had initiated egg laying. In the remaining trials, the male was left with the female during egg laying. In the interval between the end of egg laying and the start of hatching (i.e., 54–66h after pairing), we moved the remaining parents and the prepared carcass to a new box with fresh soil, while the eggs were left to develop in the original box. The larvae hatching from these eggs were then used to generate experimental foster broods. To ensure that we had an ample supply of foster larvae to generate the experimental broods, we set up additional donor pairs for breeding on the same day as the experimental pairs. We also set up some additional donor pairs over the consecutive two days. As soon as possible after their own larvae began to hatch, we provided breeding beetles with experimental foster broods that differed with respect to hatching spread (Smiseth & Morgan, 2009). The experimental broods were comprised of larvae that were unrelated to the foster parents and that were derived from up to four different donor females. The larvae were newly hatched and had not previously received any parental care from other individuals. Caring parents always received a total of 20 larvae, which is similar to the mean brood size of 21 larvae in this species (Smiseth & Moore, 2002). We weighed the larvae before placing them on the carcass as a measure of prenatal maternal investment. We generated synchronous broods by providing parents with 20 larvae at the same time. We generated asynchronous broods by providing parents with 10 larvae initially and then an additional 10 larvae 24 hours later. Finally, we generated highly asynchronous broods by providing parents with 10 initial larvae followed by 10 additional larvae 48 hours later. Thus, synchronous broods had a hatching spread of 0h, while asynchronous broods had a hatching spread of 24h and highly asynchronous broods

had a hatching spread of 48h. This is within the natural variation of hatching spread, which can extend up to 56 hours in this species with a mean of around 30 hours (Smiseth *et al.*, 2006). The total sample size in the experiment was n=126. The sample sizes for each treatment were as follows: n=20 for synchronous brood with male present, n=22 for synchronous brood with male absent, n=20 for asynchronous brood with male present, n=22 for asynchronous brood with male absent, n=22 for highly asynchronous brood with male present, and n=20 for highly asynchronous brood with male absent.

We used the amount of time that each parent spent with the brood from the arrival of the first larvae as a proxy for the amount of care they provided (Boncoraglio & Kilner, 2012; Smith *et al.*, 2014). We checked each box twice a day (at 09:00h and 17:15h) to determine whether the parent was present or absent from the brood chamber. If a parent was absent in two consecutive observations, we regarded it as having deserted the brood (Smith *et al.*, 2014). Once the deserting parent had been removed from the box, we weighed it to record its post-breeding body mass. If parents did not desert the brood before larval dispersal (defined as when the majority of larvae left the carcass), we weighed and removed them at the time when the larvae dispersed from the carcass. We placed all parents in individual boxes upon removal from the breeding box, and fed them small pieces of organic beef twice a week. We recorded the number of larvae dispersing from each brood and weighed the entire brood to obtain the total dispersing brood mass, from which we calculated the average larval mass. We then placed the larvae in a box (17cm x 12cm x 6cm) filled with soil and allowed them to eclose. As keeping all offspring would amount to an excessive workload, we randomly selected one male and one female offspring from each brood upon eclosion and retained them to record potential effects on lifespan. We recorded the sex and pronotum width of the other offspring. We checked parents and retained offspring at least three times a week to obtain the approximate age of death, and measured their pronotum widths using a Mitutoyo Absolute Digimatic calliper.

Statistical analyses

We carried out the statistical analyses in R (R Core Team, 2014). We selected model families and link functions based on graphical model validation and AIC values where appropriate. We carried out model refinement through backwards stepwise deletion using the drop1 function (p -values based on F or Chi statistics). To analyse the results of Experiment 1, we constructed generalised linear models to investigate the effect of male removal on laying spread (Gamma family, inverse link function) and laying skew (Gaussian family, identity link function). We used Kendall's Tau correlation to test for a correlation between laying spread and laying skew. We constructed generalised linear models to investigate the influence of hatching pattern on parental care and aspects of parent and offspring fitness studied in Experiment 2. Table 1 shows the full models and the model family and link function used in each model. We used Kendall's Tau correlation to test for a correlation between male presence and female presence. We used Wilcoxon signed ranks test to determine whether female presence was affected by removal of the male. We also used Wilcoxon signed ranks tests to compare female age at death and male age at death between treatments where the male was removed or was allowed to remain with the brood. Finally, we compared total parental presence between hatching patterns using a Kruskal-Wallis test.

RESULTS

Experiment 1

In contrast to what we predicted if females facultatively adjusted their egg laying to the presence or absence of the male, the removal of the male before oviposition did not affect average laying spread ($F_{1,48}=0.09$, $p=0.768$). Laying skew was also not affected by male removal ($F_{1,48}=2.60$, $p=0.1137$) and there was no correlation between laying spread and laying skew ($z=0.30$, $p=0.763$). Laying spread was greater for larger clutches ($F_{1,48}=21.57$, $p<0.0001$) and there was a non-significant trend towards a greater laying spread when oviposition commenced earlier ($F_{1,48}=4.00$, $p=0.0514$).

Experiment 2

Consistent with what we predicted, the hatching pattern had a significant effect on the amount of time that the male was caring for the brood (the number of observations the parent was present out of total number of observations when there were larvae on the carcass) ($t_{58}=3.18$, $p=0.0024$). The male remained for longest when caring for highly asynchronous broods (Fig. 1a). In contrast, the female deserted highly asynchronous broods sooner ($F_{1,60}=5.41$, $p=0.0234$, Fig.1b). Although the amount of time the male was present was highly negatively correlated with the amount of time the female was present ($z=-3.27$, $p=0.0011$), the amount of time the female was present was not affected by the removal of the male before oviposition ($W=2246$, $p=0.171$). This suggests that the male responds by delaying his desertion of the brood when the female deserts first, while the female's decision was not affected by male desertion. Furthermore, the male responded differently to female desertion depending on the hatching pattern (interaction between hatching pattern and amount of time females provided care: $F_{1,58}=9.68$, $p=0.0029$); the male responded more strongly to female desertion when he was caring for highly asynchronous broods. Despite this, the total amount of presence by male and female parents was similar across all three hatching patterns ($\chi^2=4.74$, $p=0.0934$). There was no effect of female pronotum width ($F_{1,60}=3.01$, $p=0.0938$) or male pronotum width ($F_{1,58}=0.00$, $p=0.997$) on the duration of care provided.

In contrast to what we predicted, we found no evidence that a reduction in the duration of female care led to an increase in the female's own lifespan or a reduction in her loss in body mass during breeding. The female's adult lifespan (number of days from eclosion to death) was not affected by the hatching pattern ($F_{1,123}=1.16$, $p=0.283$). Furthermore, female lifespan was not affected by the absolute duration of time the female spent with the brood ($F_{1,123}=0.16$, $p=0.695$). Similarly, male lifespan was not affected by the hatching pattern ($F_{1,60}=0.38$, $p=0.541$) or the amount of time spent with the brood ($F_{1,60}=0.06$, $p=0.814$). Females had a significantly longer lifespan than the males ($W=4492$, $p<0.0001$). Female lifespan was not affected by whether the male was present or absent ($W=1934$, $p=0.931$), the males survived slightly longer when allowed to remain with the brood ($W=1519$, $p=0.0322$). We found that most parents (95% of all parents) gained mass during

the breeding attempt. Mass change was not affected by hatching pattern ($F_{1,185}=0.41$, $p=0.521$), sex ($F_{1,185}=0.18$, $p=0.6697$), or the interaction between sex and hatching pattern ($F_{1,185}=0.01$, $p=0.907$). However, mass change was greater when parents remained with the brood for longer ($F_{1,185}=6.44$, $p=0.0120$) and was also greater for parents that initially had a lower body mass ($F_{1,185}=11.15$, $p=0.0010$).

Hatching patterns influenced larval survival as a greater number of larvae survived to dispersal in synchronous or asynchronous broods than in highly asynchronous broods ($\chi^2_{1,123}=12.76$, $p=0.0004$, Fig. 2). Larval survival was not affected by male removal ($\chi^2_{1,123}=0.66$, $p=0.418$). However, the number of surviving larvae was greater when the larvae had a greater initial mass at the time when they were introduced to the carcass ($\chi^2_{1,123}=26.40$, $p<0.0001$). The duration of larval development was also affected by hatching pattern ($F_{1,124}=22.12$, $p<0.0001$) as highly asynchronous broods took on average 0.63 days longer to reach dispersal than synchronous broods. Offspring lifespan was not affected by hatching pattern ($F_{1,248}=0.80$, $p=0.371$), male removal ($F_{1,248}=0.021$, $p=0.886$), the total duration of parental care ($F_{1,248}=0.29$, $p=0.588$), the offspring's sex ($F_{1,248}=3.28$, $p=0.071$), or offspring pronotum width ($F_{1,246}=0.33$, $p=0.565$). The mean mass of a larva at dispersal decreased with increasing hatching spread ($F_{1,123}=36.05$, $p<0.0001$, Fig. 3) and increased with increasing total duration of parental presence ($F_{1,123}=14.63$, $p=0.0002$). However, mean larval mass was not affected by male removal ($F_{1,123}=0.16$, $p=0.694$). Similarly, the mean pronotum width of the offspring from each brood also decreased with increasing hatching spread ($F_{1,124}=22.50$, $p<0.0001$) and was not affected by male removal, although there was a non-significant trend towards greater offspring pronotum widths when the male was allowed to remain with the brood ($F_{1,124}=3.91$, $p=0.0502$). The standard deviation in offspring pronotum width increased with increasing hatching spread ($F_{1,124}=79.10$, $p<0.0001$).

DISCUSSION

In Experiment 1, we found that laying spread and laying skew were similar regardless of whether the male was experimentally removed or allowed to remain with the female during oviposition. This finding suggests that females do not adjust hatching patterns facultatively in response to male removal in *N. vespilloides*, and thus that the degree of asynchronous hatching represents an evolutionary response to sexual conflict over parental care. We are unaware of any evidence from the literature showing that females adjust hatching patterns facultatively in response to the absence or removal of the male. The absence of evidence for facultative responses in *N. vespilloides* and birds might reflect that there has not been strong selection on females to adjust hatching patterns depending on whether the male is present or absent, possibly reflecting that females normally are assisted by a male partner (Scott, 1998; Cockburn, 2006). If the hatching pattern is an evolutionary response to sexual conflict over parental care, we might expect the optimal hatching pattern for the female to depend on male parental effort and the optimal parental effort for the male to depend on the hatching pattern. The outcome of this co-evolutionary process might be for females to evolve a hatching pattern that is associated with an evolutionary increase in male parental effort to relative to female parental effort.

In Experiment 2, we found that males remained for longer when caring for highly asynchronous broods than when caring for asynchronous and synchronous broods, while females in contrast deserted earlier when caring for highly asynchronous broods. This difference between males and females is consistent with the prediction of the sexual conflict hypothesis (Slagsvold & Lifjeld, 1989), and suggests that females could increase male contributions to parental care by laying the eggs more asynchronously. We suggest two possible mechanisms for the observed effect of hatching pattern on the duration of paternal care. Firstly, males may prolong their involvement in care when caring for highly asynchronous broods in response to the female deserting earlier. In support of this suggestion, we found that males remained with the brood for longer when their partner deserted earlier, and similar results showing that males adjust their contribution to the absence of the female have been found in previous studies on the amount of care

(Fetherston *et al.*, 1994; Smiseth & Moore, 2004; Rauter & Moore, 2004; Smiseth *et al.*, 2005; Suzuki & Nagano, 2009; Smith *et al.*, 2014). We found that males responded more strongly to female desertion in highly asynchronous broods, but that the total duration of parental care was similar across all hatching patterns. Males may respond by staying for longer should the female desert early on in order to prevent conspecifics from usurping the carcass and killing the brood (Trumbo, 1990). Secondly, our results may reflect that males reduce their effort later if they contributed more towards care early on (Amundsen, 1999). Smiseth and Morgan (2009) found that the peak in brood demand is significantly higher in synchronous broods than in highly asynchronous broods. Thus, males may initially contribute more towards parental care when caring for synchronous broods while there is a high demand, and may desert the brood earlier as a consequence of their higher initial contribution. If so, males might potentially be contributing a similar amount of care towards synchronous and asynchronous broods by remaining for longer but providing lower levels of care in the latter broods. We have no information on the amount of care given that we used residency time as a proxy for parental effort instead of behavioural observations. Thus, further work is now needed to examine the mechanisms whereby hatching patterns influence the duration of paternal care.

We found that the duration of female care was not influenced by the removal of the male before larvae were present on the carcass, suggesting that females do not adjust their timing of desertion in response to male behaviour. Similar results showing that females do not respond to male removal have been found in previous studies on *N. vespilloides* (Smiseth *et al.*, 2005) and the closely related *N. orbicollis* (Rauter & Moore, 2004). We also found that females deserted highly asynchronous broods earlier than asynchronous or synchronous broods. This finding might reflect that females value highly asynchronous broods less highly given that we also found that these broods produce fewer and smaller surviving larvae. Previous work shows that females adjust their provisioning behaviour in response to changes in the demand of the older larvae in the brood rather than the entire brood (Smiseth & Morgan, 2009). Thus, females may be more sensitive to the requirements

of older larvae, in which case they might desert the brood depending on the age of the older larvae in the brood rather than the average age of the brood. The greater duration of female care seen in synchronous broods with 20 older larvae than in asynchronous and highly asynchronous broods with 10 older larvae is consistent with this suggestion. It is not known whether males are more responsive to the needs of older larvae than younger larvae, but given that they provide less care overall than females, they may not be under strong selection to discriminate between different-aged larvae. Further work is needed to examine whether there is a difference in how males and females respond to the demand of the older larvae relative to the entire brood.

Our study shows that asynchronous hatching had detrimental effects on the offspring's fitness. Firstly, we found that larval survival was lowest in highly asynchronous broods, as previously reported by Smiseth and Morgan (2009). This finding shows that high levels of hatching asynchrony increase the offspring's mortality. Secondly, we found that mean larval mass at dispersal and offspring pronotum width at eclosion were lower in highly asynchronous broods, which contrasts with previous studies reporting no effect of hatching patterns on larval mass at dispersal (Smiseth *et al.*, 2008; Smiseth & Morgan, 2009). Such reductions in offspring size should have detrimental fitness consequences given that smaller offspring develop into smaller adults (Lock *et al.*, 2004), and that smaller adults are less likely to be successful in competition for breeding resources (Otronen, 1988; Scott & Traniello, 1990; Trumbo, 1991; Robertson, 1993). Thirdly, we found that the duration of larval development was longer for highly asynchronous broods, potentially increasing the vulnerability of the brood to predation or infanticidal intruders. The detrimental effects of asynchronous hatching on offspring survival and growth are likely to be the outcome of asymmetric sibling competition caused by asynchronous hatching. Smiseth *et al.* (2007) found that older larvae in asynchronous broods grow better than younger larvae as long as the parents provide care, suggesting that parental care somehow exacerbates asymmetric sibling competition. Previous work on burying beetles suggests that older larvae consistently have higher survival and greater body mass than younger larvae regardless of hatching

spread or skew (Takata *et al.*, 2014), reflecting that they receive more parental care (Smiseth *et al.*, 2007; Smiseth & Moore 2008; Takata *et al.*, 2013; Andrews & Smiseth, 2013). Similar detrimental effects of asymmetric sibling competition on offspring survival and growth have also been reported in birds (Clark & Wilson, 1981). These detrimental fitness consequences of asynchronous hatching have important consequences for the sexual conflict hypothesis because, although hatching asynchrony provides females with a means for increasing the male's contribution to parental care, females can only do so by also reducing their offspring's size and survival. Thus, females should be under selection to produce an intermediate hatching pattern that provides the best possible trade-off between the benefits of increased male parental effort and the costs due to reduced offspring fitness.

We found that mean larval mass was greater when parents provided care for longer. Previous work has found that parental care improves offspring growth, particularly during the early stages of larval development (Eggert *et al.*, 1998; Smiseth *et al.*, 2003). Thus, caring for the brood for longer has positive effects on the offspring's fitness. The fact that one of the parents often deserts the brood prematurely therefore suggests that there must be some cost of providing care, such as reduced lifespan or increased loss of body mass, or that there are some benefits of deserting the brood, such as increased opportunities for breeding (Royle *et al.*, 2012). If the sexual conflict hypothesis is to be supported, the benefits that the female gain from increasing the male's contribution towards care for highly asynchronous broods, thereby allowing the female to reduce her own costs of care, should outweigh the detrimental effect of reduced offspring fitness. Currently, it is unclear what costs parents incur from providing care in burying beetles. There does not appear to be an immediate physiological cost of breeding given that almost all parents in our study gained mass during breeding – a result that also has been found in the closely related *N. orbicollis* (Scott & Traniello, 1990) – and that parents that remained with the brood for longer gained proportionally more mass. Furthermore, we did not find any long-term cost of caring, as there were no effects of the hatching pattern or the duration of parental care on female lifespan. In contrast to what was found by Boncoraglio and Kilner (2012), we found no effect of male presence after hatching

on female lifespan. This may reflect that the benefit to females of being assisted by a male depends on the ecological context. For example, male presence could be detrimental if there is competition for food between parents and offspring on small carcasses because dispersing brood mass is lower for broods reared by males that gain mass (Scott & Gladstein, 1993) and females sometimes even kill males on very small carcasses (Bartlett, 1988). The carcasses used in the present study were large enough to support 20 larvae and thus there were probably sufficient resources for two parents to feed without depriving the offspring of food.

Although our study did not identify a benefit to the female of reducing her duration of care, it is possible that we were unable to detect such a benefit in our laboratory experiment. For example, females may benefit from deserting earlier by reducing the risk infection by microorganisms present on the carcass. We always used fresh carcasses in our experiment, but females in the field may breed on carcasses that have begun to decompose before the start of the breeding attempt (Steiger *et al.*, 2011). Furthermore, females may benefit from deserting earlier by reducing the risk of injury or death during fights with conspecifics. We always excluded competitors in our experiments, while females in the field may face both interspecific and intraspecific competitors, which may cause injury or death. Thus, future work on the potential benefits to females from increasing the amount of male care should consider designs that mimic the harsher conditions these beetles face in the wild.

Our study is the first to test the sexual conflict hypothesis in a non-avian species. We found some support for the sexual conflict hypothesis in the burying beetle *N. vespilloides*. As predicted, we found that males and females responded differently to hatching patterns: males provided care for longer in highly asynchronous broods whereas the opposite was true of females. Our findings suggest that asynchronous hatching may play a role in the resolution of sexual conflict over parental care in *N. vespilloides*. However, we did not find any evidence that females benefitted from reducing their duration of care, and we found costs of high levels of hatching asynchrony in terms of reduced larval growth and survival. We argue that hatching asynchrony would only be a viable strategy for females to increase

the male's contribution to care if the benefits to females from reducing their own costs of care outweigh the costs of reduced offspring fitness. We recommend that future studies on the sexual conflict hypothesis recognise the importance of assessing fitness consequences for parents and offspring in addition to studying changes in each parent's contribution towards parental care.

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Figure legends

Figure 1: Duration of time spent with the brood as a proportion of the total time larvae were on the carcass for synchronous, asynchronous and highly asynchronous broods. a) Mean duration of male presence with the brood. b) Mean duration female presence with the brood. Error bars indicate ± 1 s.e.

Figure 2: Mean number of larvae dispersing from the carcass for synchronous, asynchronous and highly asynchronous broods. Error bars indicate ± 1 s.e.

Figure 3: Mean mass of a larva at dispersal (total brood mass divided by number of larvae) for synchronous, asynchronous and highly asynchronous broods. Error bars indicate ± 1 s.e.

Table 1: Summary of generalised linear models showing all terms included in the full model, and model families and link functions.

Response variable	Full model	Family	Link function
Female presence	Hatching pattern+Carcass mass+Female body size+Female age	Quasi	$1/\mu^2$
Male presence	Hatching pattern+Carcass mass+Female body size+Female age	Quasi	Identity
Parent proportional mass change	Hatching pattern+Sex+Sex:Hatching pattern+Time present+Carcass mass+Initial mass	Gaussian	Identity
Female lifespan	Hatching pattern+Time present+Body size+Age	Gamma	Identity
Male lifespan	Hatching pattern+Time present+Body size+Age	Gamma	Identity
Number of larvae dispersing	Hatching pattern+Total parental presence+Male presence+Larval development time+Carcass mass+Initial mass	Poisson	Identity
Mean larval mass at dispersal	Hatching pattern+Male presence+Hatching pattern:Male presence+Total parental presence+Larval development time +Carcass mass	Inverse Gaussian	Identity
Mean offspring pronotum width	Hatching pattern+Male presence+Hatching pattern:Male presence+Carcass mass	Gaussian	Inverse
Standard deviation in offspring pronotum width	Hatching pattern+Male presence+Hatching pattern:Male presence+Carcass mass	Inverse Gaussian	Log

Duration of larval development	Hatching pattern+Male presence+Carcass mass	Gaussian	Identity
Offspring age at death	Hatching pattern+Male presence+Body size+Sex+Total parental presence	Gamma	Inverse

